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(57) Abstract

The present invention provides isolated and purified polynucleotides that encode HVEM of mammalian origin, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making HVEM using those polynucleotides and vectors, and isolated and purified HVEM.

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Herpes Simplex Virus 1 Entry into Cells Mediated by a Novel Member of the TNF/NGF Receptor Family

Cross Reference to Related Applications

This application is a continuation-in-part of United States Patent application Serial No. 08/509,024 filed on July 28, 1995, the disclosure of which is incorporated herein by reference.

Technical Field of the Invention

The field of this invention is a herpes simplex virus cellular mediator (HVEM). More particularly, the field of the present invention is recombinant mammalian HVEM, polynucleotides encoding that HVEM, and methods of making recombinant HVEM.

Background of the Invention

Herpes simplex viruses (HSV) are human members of the neurotropic subgroup (alphaherpesviruses) of the herpesvirus family. Infections with HSV type 1 (HSV-1), HSV type 2 (HSV-2) or both are highly prevalent in human populations. The usual manifestations of disease (reviewed by Corey and Spear, 1988) are mucocutaneous lesions of the mouth, face, eyes or genitalia. The facile spread of virus to the peripheral nervous system permits the establishment of life-long latent infections in neurons. Latent virus can be activated to cause recurrent lesions at or near the site of the primary lesions. In addition, the virus can spread via neural routes to the central nervous system to cause meningitis or encephalitis. Viral replication in the natural host usually remains localized to cells of the epidermis and peripheral nervous system, except in newborn infants, who are more prone to disseminated infection. Differentiated cell types probably vary in their susceptibility to HSV entry. However, cultured cells of various types from many animal species are susceptible to HSV infection, indicating that cell receptors for viral entry may be highly conserved or multiple in number and are usually expressed on rapidly dividing cells.

There are differences in the natural history of recurrent disease, which depend on the site of infection and serotype, despite the fact that HSV strains of either serotype can cause clinically indistinguishable lesions at any of the usual sites. For example, HSV-2 strains are more likely than HSV-1 strains to cause recurrent disease at genital sites of infection whereas the converse is true for oral sites of infection (Lafferty et al., 1987). The two serotypes may differ in some

aspects of regulation of the latent state at different anatomic sites (reviewed by Fraser and Valyi-Nagy, 1993). In addition, the two serotypes may differ in some of the requirements for viral entry into cells (Vahlne et al., 1979; Gerber et al., 1995; Herold et al., 1996). For example, Chinese hamster ovary cells (CHO-K1) are resistant to the entry of many HSV-1 strains but are more susceptible to the entry of HSV-2 strains (Shieh et al., 1992).

The binding and penetration phases of viral entry into cells can be experimentally dissociated. The binding of HSV-1 or HSV-2 to cells is mediated by the interaction of viral envelope glycoproteins (gB and/or gC) with glycosaminoglycan chains (GAGs) of cell surface proteoglycans (reviewed by Spear, 1993). The particular structural features recognized on the heterogeneous GAG chains differ for HSV-1 and HSV-2 (Herold et al., 1996). These viruses penetrate into cells by pH-independent fusion of the virion envelope with a cell membrane, either the plasma membrane or an early endosome (Wittels and Spear, 1991). The viral glycoproteins required for penetration include gB, gD, and gH-gL hetero-oligomers (Sarmiento et al., 1979; Cai et al., 1988; Ligas and Johnson, 1988; Forrester et al., 1992; Roop et al., 1993). Binding of HSV-1 or HSV-2 to cells is not sufficient to mediate penetration since certain cell types, such as swine testis (ST) or CHO-K1 cells, can bind virus efficiently but restrict viral entry. ST cells are resistant to the entry of both HSV-1 and HSV-2 (Subramanian et al., 1994; Subramanian et al., 1995) and, as mentioned above, CHO-K1 cells are much more resistant to HSV-1 entry than to HSV-2 entry (Shieh et al., 1992). Previous studies indicated that the derivation of stable cell lines from Chinese hamster tissues correlated with increased resistance of the cell lines to HSV-1 infection and that presence of human chromosomes in Chinese hamster lung-human cell hybrids was associated with enhanced viral replication (Francke and Francke, 1981).

These observations suggested that expression of the appropriate human gene in CHO-K1 cells might restore susceptibility to HSV entry. By screening a human cDNA expression library for genes capable of converting CHO-K1 cells from resistance to susceptibility to HSV-1 entry, a previously undescribed member of the tumor necrosis factor/nerve growth factor (TNF/NGF) receptor family has been identified. This receptor can mediate the efficient entry of HSV-1 strains into CHO-K1 cells and ST cells and can also enhance the entry of HSV-2 strains into CHO-K1 cells.

Brief Summary of the Invention

In one aspect, the present invention provides an isolated and purified polynucleotide comprising a nucleotide sequence consisting essentially of the nucleotide of SEO ID NO:1 from about nucleotide position 294 to about nucleotide position 1142; (b) sequences that are complementary to the sequences of (a), and (c) sequences that, when expressed, encode a polypeptide encoded by a sequence of (a). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule. A preferred polynucleotide is SEQ ID NO:1.

In another embodiment, a DNA molecule of the present invention is contained in an expression vector. The expression vector preferably further comprises an enhancer-promoter operatively linked to the polynucleotide. In an especially preferred embodiment, the DNA molecule has the nucleotide sequence of SEQ ID NO:1 from about nucleotide position 294 to about nucleotide position 1142.

In another aspect, the present invention provides an oligonucleotide of from about 15 to about 50 nucleotides containing a nucleotide sequence of at least 15 nucleotides that is identical or complementary to a contiguous sequence of a polynucleotide of this invention. A preferred oligonucleotide is an antisense oligonucleotide that is complementary to a portion of the polynucleotide of SEQ ID NO:1.

The present invention also provides a pharmaceutical composition comprising a polypeptide or an antisense oligonucleotide of this invention and a physiologically acceptable diluent.

In another aspect, the present invention provides an HVEM polypeptide of mammalian origin. In one embodiment, that HVEM is an isolated and purified polypeptide of about 283 amino acid residues and comprises the amino acid residue sequence of SEQ ID NO:2. More preferably, an HVEM of the present invention is a recombinant human HVEM

In another aspect, the present invention provides a process of making HVEM comprising transforming a host cell with an expression vector that comprises a polynucleotide of the present invention, maintaining the transformed cell for a period of time sufficient for expression of the HVEM and recovering the

HVEM. Preferably, the host cell is an eukaryotic host cell such as a mammalian cell or a bacterial cell. An especially preferred host cell is a mammalian ovarian cell. The present invention also provides an HVEM made by a process of this invention. A preferred such HVEM is recombinant human HVEM.

The present invention still further provides for a host cell transformed with a polynucleotide or expression vector of this invention. Preferably, the host cell is a mammalian cell such as an ovarian cell.

Brief Description of the Drawings

FIG. 1 shows entry of HSV-1 into HeLa cells, CHO-K1 cells and transfected CHO-K1 cells. CHO-K1 cells or HeLa cells plated in 96-well dishes were exposed to 50 µl of HSV-1(KOS)gL86 at the input doses indicated. Infection was quantitated by monitoring the expression of β-galactosidase from the input viral genome. Six hours after the addition of virus, the cells were solubilized with detergent and β-galactosidase substrate was added. The colored reaction product was quantitated by spectrometry at several time points after the addition of substrate to define the interval over which the generation of product was linear with time. Each point represents the mean of triplicate determinations. The individual values were within 10% of the mean. HeLa cells (open circles) and CHO-K1 cells (closed circles); values obtained 4 hr after substrate addition.

FIG. 2 shows the nucleotide sequence of the HVEM cDNA (SEQ ID NO:1) and amino acid sequence (SEQ ID NO:2) of the open reading frame. The nucleotide sequence of the cDNA insert of pBEC580 was determined as shown. Analysis of the sequence revealed the presence of a single open reading frame, the translation of which is shown below the nucleotide sequence. Features of the 283-amino acid open reading frame include a signal peptide (dotted underline), two potential sites for the addition of N-linked carbohydrate (single underline) and a hydrophobic region predicted to be a membrane-spanning domain (double underline). Also indicated by the patterned bars under the amino acid sequence are the three complete and one partial cysteine-rich repeats characteristic of members of the TNF/NGF receptor family. Asterisks show the positions of amino acids that are highly conserved in the family. The arrow after Cys185 indicates the last amino acid of HVEM present in the fusion protein, HVEM:Fc. The arrow after Ala257 indicates the last amino acid of HVEM present in the epitope-tagged truncated protein, HVEM257-Flu. The sequence has been deposited in GenBank Database.

FIG. 3, having panels A and B, shows enhanced entry of HSV-1(KOS) into HVEM-expressing cell lines derived from CHO-K1 and ST cells. Several HVEM-expressing and control cell lines were obtained by transfection of CHO-K1 cells or ST cells with pBEC10 or the control plasmid, pcDNA3, followed by selection for stable maintenance of the plasmid. Several representative clones were plated in 96-well plates and exposed to HSV-1(KOS)gL86 at the input doses indicated. Six hours later viral entry was quantitated by assay for β-galactosidase activity as described in the legend to FIG. 1. (A) Clones derived from CHO-K1 cells; (B) clones derived from ST cells. CHO-HVEM11 and ST-HVEM1 (open circles), CHO-HVEM9 and ST-HVEM22 (closed circles) and CHO-HVEM12 and ST-HVEM2 (open squares) were isolated after transfection with the HVEM-expressing plasmid pBEC10. CHO-C8 and ST-C8 (closed squares) were isolated after transfection with the control plasmid pcDNA3.

FIG. 4, having panels A and B, shows the effects of anti-HVEM antibodies and HVEM:Fc on HSV-1(KOS) infection of HVEM-expressing cells. CHO-HVEM12 cells or ST-HVEM1 cells were plated in 96-well dishes. In the top panels the cells were exposed to pre-immune or immune rabbit serum at the dilutions indicated for 30 minutes at 37oC. Various concentrations of HSV-1(KOS)gL86 were then added in 1/5 volume and incubation continued for 2 hours. In the bottom panels virus was mixed with various concentrations of HVEM:Fc or normal rabbit IgG and incubated for 30 minutes at 37oC. The mixtures were then added to washed cells and incubation continued for 2 hours. The virus-serum or virus-HVEM:Fc mixtures were then removed and the cells exposed to low pH buffer to inactivate any virus that had bound to cells but not yet penetrated. After washing the cells and replacement of medium, incubation was continued for an additional 4 hours before lysis of the cells and addition of β-galactosidase substrate. The amount of virus added was 107 PFU per well for the top panels and 106 PFU per well for the bottom panels.

FIG. 5, having panels A and B, shows enhanced entry of HSV-1 and HSV-2 strains into HVEM-expressing CHO-IEβ8 cells. CHO-IEβ8 cells were transfected with the HVEM-expressing plasmid pBEC10 (diagonal-hatched bars) or the control plasmid pcDNA3 (open bars). At 24 hours after transfection the cells were replated in 96-well dishes and, 24 hours later, were exposed to various input doses of each of the virus strains indicated. Six hours later the cells were lysed and β-galactosidase substrate added for the quantitation of viral entry. The

results presented are for a single input dose of virus (30,000 PFU added per well) in the linear range of the dose response curve.

FIG. 6 shows a restriction map of plasmid pBEC580.

FIG. 7 shows a restriction map of plasmid pBEC10.

FIG. 8 shows a restriction map of plasmid pBL58.

Detailed Description of the Invention

I. The Invention

The present invention provides isolated and purified polynucleotides that encode HVEM of mammalian origin, expression vectors containing those polynucleotides, host cells transformed with those expression vectors, a process of making HVEM using those polynucleotides and vectors, and isolated and purified HVEM.

II. HVEM Polynucleotides

In one aspect, the present invention provides an isolated and purified polynucleotide that encodes an HVEM polypeptide of mammalian origin.

A polynucleotide of the present invention that encodes HVEM is an isolated and purified polynucleotide that comprises a nucleotide sequence consisting essentially of the nucleotide sequence of SEQ ID NO:1 from about nucleotide position 294 to about nucleotide position 1142 of SEQ ID NO:1, (b) sequences that are complementary to the sequences of (a), and (c) sequences that, when expressed, encode a polypeptide encoded by the sequences of (a). A preferred polynucleotide is a DNA molecule. In another embodiment, the polynucleotide is an RNA molecule.

A nucleotide sequence and deduced amino acid residue sequence of human HVEM are set forth in FIG. 2. The nucleotide sequence of SEQ ID NO:1 in FIG. 2 is a full length DNA clone of human HVEM. SEQ ID NO:2 in FIG. 2 is the deduced amino acid residue sequence of that clone.

The present invention also contemplates DNA sequences which hybridize under stringent hybridization conditions to the DNA sequences set forth above. Stringent hybridization conditions are well known in the art and define a degree

of sequence identity greater than about 70%-80%. The present invention also contemplates naturally occurring allelic variations and mutations of the DNA sequences set forth above so long as those variations and mutations code, on expression, for an HVEM of this invention as set forth hereinafter.

As set forth above, SEQ ID NO:1, is a full length cDNA clone of human HVEM. As is well known in the art, because of the degeneracy of the genetic code, there are numerous other DNA and RNA molecules that can code for the same polypeptide as those encoded by SEQ ID NO:1. The present invention, therefore, contemplates those other DNA and RNA molecules which, on expression, encode for the polypeptide encoded by SEQ ID NO:1. Having identified the amino acid residue sequence of HVEM, and with knowledge of all triplet codons for each particular amino acid residue, it is possible to describe all such encoding RNA and DNA sequences. DNA and RNA molecules other than those specifically disclosed herein and, which molecules are characterized simply by a change in a codon for a particular amino acid are within the scope of this invention.

A Table of codons representing particular amino acids is set forth below in Table 1.

TABLE 1

First position (5' end)	Second Position				Third position (3' end)
	T/U	C	A	G	
T/U	Phe	Ser	Tyr	Cys	T/U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	T/U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	T/U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	T/U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A

Val	Ala	Glu	Gly	G

A simple change in a codon for the same amino acid residue within a polynucleotide will not change the structure of the encoded polypeptide. By way of example, it can be seen from SEQ ID NO:1 (See FIG. 2) that a CCT codon for proline exists at nucleotide positions 300-302. It can also be seen from that same sequence, however, that proline can be encoded by a CCC codon (See e.g., nucleotide positions 321-323). Substitution of the latter CCC codon for proline with the CCT codon for proline, or vice versa, does not substantially alter the DNA sequence of SEQ ID NO:1 and results in expression of the same polypeptide. In a similar manner, substitutions of codons for other amino acid residues can be made in a like manner without departing from the true scope of the present invention.

A polynucleotide of the present invention can also be an RNA molecule. A RNA molecule contemplated by the present invention is complementary to or hybridizes under stringent conditions to any of the DNA sequences set forth above. As is well known in the art, such a RNA molecule is characterized by the base uracil in place of thymidine. Exemplary and preferred RNA molecules are mRNA molecules that encode an HVEM of this invention.

The present invention also contemplates oligonucleotides from about 15 to about 50 nucleotides in length, which oligonucleotides serve as primers and hybridization probes for the screening of DNA libraries and the identification of DNA or RNA molecules that encode HVEM. Such primers and probes are characterized in that they will hybridize to polynucleotide sequences encoding HVEM or related receptor proteins. An oligonucleotide probe or primer contains a nucleotide sequence of at least 15 nucleotides that is identical to or complementary to a contiguous sequence of an HVEM polynucleotide of the present invention. Thus, where an oligonucleotide probe is 25 nucleotides in length, at least 15 of those nucleotides are identical or complementary to a sequence of contiguous nucleotides of an HVEM polynucleotide of the present invention. Exemplary HVEM polynucleotides of the present invention are set forth above.

A preferred oligonucleotide is an antisense oligonucleotide. The present invention provides a synthetic antisense oligonucleotide of less than about 50 nucleotides, preferably less than about 35 nucleotides, more preferably less than about 25 nucleotides and most preferably less than about 20 nucleotides. An

antisense oligonucleotide of the present invention is directed against a DNA or RNA molecule that encodes HVEM. Preferably, the antisense oligonucleotide is directed against the protein translational initiation site or the transcriptional start site. In accordance with this preferred embodiment, an antisense molecule is directed against a region of SEQ. ID NO:1 from about nucleotide position 254 to about nucleotide position 334. It is understood by one of ordinary skill in the art that an antisense oligonucleotide can be directed either against a DNA or RNA sequence that encodes a specific target. Thus, an antisense oligonucleotide of the present invention can also be directed against polynucleotides that are complementary to those shown in SEQ. ID NO:1 as well as the equivalent RNA molecules.

Preferably, the nucleotides of an antisense oligonucleotide are linked by pseudophosphate bonds that are resistant to cleavage by exonuclease or endonuclease enzymes. Preferably the pseudophosphate bonds are phosphorothioate bonds. By replacing a phosphodiester bond with one that is resistant to the action of exo-and/or endonuclease, the stability of the nucleic acid in the presence of those enzymes is increased. As used herein, pseudophosphate bonds include, but are not limited to, methylphosphonate, phosphomorpholidate, phosphorothioate, phosphorodithioate and phosphoroselenoate bonds.

An oligonucleotide primer or probe, as well as an antisense oligonucleotide of the present invention can be prepared using standard procedures well known in the art. A preferred method of polynucleotide synthesis is via cyanoethyl phosphoramidite chemistry. A detailed description of the preparation, isolation and purification of polynucleotides encoding human HVEM is set forth below.

The CHO-K1 cell line is particularly resistant to the entry of HSV-1 strain KOS (Shieh et al., 1992), a wild-type strain that has been studied and characterized in a number of laboratories. A β-galactosidase-expressing version of this strain, designated HSV-1(KOS)gL86 was used to monitor entry of the virus into cells by quantitation of enzyme activity. Production of β-galactosidase from the input viral genome signals that the virus has entered the cell, released its genome to the nucleus and activated the constitutive promoter driving β-galactosidase expression. FIG. 1 shows that HeLa (human) cells were susceptible to infection by this virus whereas the CHO-K1 cells were highly resistant. To achieve equivalent levels of infection and β-galactosidase

expression required 1000 times more input virus for CHO-K1 cells than for HeLa cells. Concentrations of input virus sufficient to infect 100% of HeLa cells left the CHO-K1 cells almost totally uninfected as assessed by use of X-gal substrate, which stains HSV-1(KOS)gL86-infected cells blue.

As set forth in detail hereinafter in the Examples, the strategy employed to isolate a human cDNA that could enhance HSV-1 entry into CHO-K1 cells combined transient expression with exposure of transfected cells to HSV-1(KOS)gL86. Plasmid DNAs prepared from pools of a HeLa cell cDNA library were transfected into the CHO-K1 cells. A pool containing a positive cDNA was identified by detection of 20-30 blue cells per monolayer (35 mm diameter) after challenge of the transfected cells with HSV-1(KOS)gL86 and staining with X-gal. This pool was subdivided by an iterative process to identify individual plasmid clones with the desired phenotype.

Two plasmid clones which could render transfected CHO-K1 cells susceptible to HSV-1(KOS)gL86 entry were isolated. In transient expression experiments, CHO-K1 cells transfected with pBEC580 or pBEC748 were readily infected by HSV-1(KOS)gL86. The fraction of cells infected was comparable to the transfection efficiency, indicating that every transfected cells was probably highly susceptible to viral entry. In contrast, CHO-K1 cells transfected with a control plasmid were completely resistant to HSV-1(KOS)gL86 infection. Nucleotide sequencing and restriction endonuclease mapping revealed that the two plasmids, pBEC580 and pBEC748, contained the same cDNA insert.

Nucleotide sequencing of the cDNA insert revealed a 1724 bp cDNA (SEQ ID NO:1) encoding a single open reading frame of 283 amino acids (FIG. 2). The protein product, designated HVEM, has an N-terminal domain that can serve as a signal peptide, two potential sites for the addition of N-linked glycans, and a hydrophobic region extending from amino acids 203 to 225 that is a probable membrane-spanning domain. Thus, the protein has characteristics of a typical type I membrane glycoprotein.

To determine whether HVEM-homologous DNA sequences were present in human cells and in cells of other species, Southern analyses were performed with genomic DNAs extracted from various cell lines. Probes made from the purified cDNA insert hybridized to genomic DNA fragments from HeLa and HEp-2 (human) cells, Vero (African green monkey) cells and CHO-HVEM12

cells, which were stably transfected with an HVEM cDNA clone, but not to genomic DNA fragments from control CHO-K1 cells. The smaller PvuII probe hybridized to a single DNA band in the digests from three different human cell lines (HT1080) and, with a weaker signal, to a single DNA band of different size in the digest from the monkey cells. The full-length EcoRI probe, which has no sites for the restriction endonuclease used to digest the cell DNAs (BamHI), hybridized to additional bands as well in both the human and monkey DNA samples. Thus, HVEM is encoded by a single-copy human gene with multiple exons and non-human primates appear to have a related gene.

To assess the expression of HVEM in human organs, labeled probes prepared from the HVEM cDNA sequence were hybridized to a Northern blot of polyadenylated RNAs extracted from several human tissues. HVEM-related RNAs could be detected in most of the samples tested, with highest levels apparently in lung, liver and kidney and least in brain. The RNA species detected were heterogeneous in size. The most abundant species was about 2 kb in size. The presence of larger species (4-5 kb) suggested that transcription of the gene for HVEM may result in multiple mRNAs. The 2 kb species could represent the mRNA from which the HVEM cDNA was copied.

Two cell lines, CHO-K1 and ST, were previously reported to be resistant to HSV-1 entry (Shieh et al., 1992; Subramanian et al., 1994). CHO-K1 cells, but not ST cells, are also resistant to the entry of a porcine alphaherpesvirus, pseudorabies virus (PRV). Both cell lines were transfected with an expression plasmid carrying the HVEM insert, or a control plasmid without the insert, to obtain stable clones of HVEM-expressing or control transfectants. The HVEM-expressing clones derived from CHO-K1 or ST cells exhibited significantly enhanced susceptibility to infection by HSV-1(KOS)gL86 (FIG. 3). The HVEM-expressing clones were 100 to 1000 times more susceptible that the control transfected clones, which were as resistant as the parental cell lines. On the other hand, the HVEM-expressing clones did not differ from the control clones in susceptibility to infection by a β-galactosidase-expressing mutant of PRV. The CHO clones, regardless of plasmid used for transfection, were resistant to PRV entry, indicating that HVEM is not a general mediator of alphaherpesvirus entry.

Experiments were done to determine whether anti-HVEM antibodies could protect stably transfected cells from HSV infection. CHO-HVEM12 cells

and ST-HVEM1 cells were exposed to various dilutions of the anti-HVEM rabbit serum or control pre-immune serum for 30 minutes prior to addition of HSV-1(KOS)gL86. As a specificity control, the ST-HVEM1 cells were challenged with PRV as well as with HSV (these cells should be susceptible to PRV independent of HVEM expression). After 2 hours of additional incubation with the serum-virus mixtures, the cells were washed and extracellular virus inactivated by exposure of the cells to a low pH buffer. Medium was replenished, incubation continued, and 6 hr after the addition of virus, β-galactosidase was quantitated to assess the number of cells infected. The anti-HVEM antiserum, but not the control serum, protected both CHO-HVEM12 cells and ST-HVEM1 cells from HSV-1(KOS)gL86 infection (FIG. 4). Antiserum dilutions of 1:810 and 1:300 inhibited virus infection of CHO-HVEM12 and ST-HVEM1 cells, respectively, by approximately 50 percent. The anti-HVEM antiserum had no effect on the ability of PRV to infect ST-HVEM1 cells, as expected.

Previous studies showed that virus binding is dependent on the presence of appropriate cell surface GAGs (WuDunn and Spear, 1989; Shieh et al., 1992; Banfield et al., 1995) and that HSV-1 apparently binds at normal levels to both CHO-K1 cells and ST cells (Shieh et al., 1992; Subramanian et al., 1994). It seemed likely that the anti-HVEM antibodies protected the cells from HSV-1 infection by binding to cell surface HVEM and interfering with viral penetration. To assess the effects of antibodies on attachment of virus to HVEM-expressing cells, the binding of radiolabeled wild-type HSV-1(KOS) to CHO-HVEM12 cells was quantitated, at several virus concentrations, in the presence of anti-HVEM serum or pre-immune serum at a 1:90 dilution. This dilution of antiserum provided complete inhibition of HSV-1 infection at all input doses of virus tested (FIG. 4). Virus binding as a function of input concentration was as previously reported for HSV-1(KOS) (Shieh et al., 1992; Herold et al., 1991) and was indistinguishable for anti-HVEM or pre-immune serum. Thus, the antibodies blocked HSV-1 infection by interfering with penetration (or possibly with events leading to uncoating and expression of the genome), and not by reducing the amount of virus bound to the cells. The antibodies also did not act by rendering the cells unresponsive to virus entry in general, or by inhibiting cell functions required for gene expression, because the antibody-treated ST-HVEM1 cells remained fully susceptible to entry of PRV and expression of β-galactosidase from the viral genome. Thus, it is probable that the anti-HVEM antibodies

blocked an interaction between cell surface HVEM and HSV-1 virions that is required for viral entry.

Since anti-HVEM antiserum could block HSV-1 infection, the possibility existed that the HVEM:Fc hybrid protein might also block infection by competing with cell surface HVEM for interactions with virus necessary for entry. To test this hypothesis, various concentrations of HVEM:Fc, or rabbit IgG as control, were incubated with HSV-1(KOS)gL86 and then the mixtures were added to CHO-HVEM12 cells or ST-HVEM1 cells to assess effects of the HVEM:Fc on viral infectivity. The results (FIG. 4) showed that HVEM:Fc, but not normal rabbit IgG, inhibited HSV-1(KOS)gL86 infection. HVEM:Fc concentrations of 1 mg and 4 mg inhibited virus infection of CHO-HVEM12 and ST-HVEM1 cells, respectively, by about 50 percent.

To determine whether HVEM-expressing cells had enhanced susceptibility to other strains of HSV besides HSV-1(KOS), experiments were carried out with a derivative of CHO-K1 cells, designated CHO-IEβ8, that was stably transfected with a plasmid carrying the E. coli *lacZ* gene under control of an HSV-1 immediate-early promoter. Expression of β-galactosidase by these cells is dependent on presence of the HSV trans-inducer designated VP16 or α-TIF (Campbell et al., 1984; Batterson and Roizman, 1983), a component of the virion that is delivered into the cell along with the nucleocapsid during viral entry. The CHO-IEβ8 cells were transfected with an HVEM-expressing or control plasmid and then challenged with various concentrations of a variety of HSV-1 and HSV-2 strains. FIG. 5 shows the results obtained with an equivalent input dose of each virus, within the linear range of the viral dose response. Expression of HVEM significantly enhanced the entry of all HSV-1 and HSV-2 strains tested, indicating that HVEM can mediate the entry of all these strains into CHO-K1 cells. The CHO-IEβ8 cells transfected with control plasmid, like the parental CHO-K1 cells (Shieh et al., 1992), were not completely resistant to the entry of all of these viruses, particularly HSV-1(MP) and HSV-2(333). This indicates that some hamster factor must be able to mediate HSV entry, albeit inefficiently and in a strain-dependent fashion. HSV-2 strains in general appear to infect CHO-K1 cells more efficiently than do HSV-1 strains (Shieh et al., 1992), one indication of the serotype-specific differences in the requirements for entry. Both HSV-1(MP) and HSV-1(KOS)804 are mutants with enhanced ability to induce cell fusion due to missense mutations at the same position in gK (Pogue-Geile and Spear, 1987; Roop et al., 1993). The syncytial mutation alone

clearly does not account for the enhanced ability of HSV-1(MP) to infect CHO cells. HSV-1(MP) has additional mutations including one that eliminates gC expression (Pogue-Geile et al., 1984), which may contribute to its complex phenotype.

HVEM-expressing CHO cells were found to be permissive for viral replication, indicating that the principal block to viral replication in CHO-K1 cells is the block in viral entry. Not unexpectedly, the amount of virus produced in HVEM-expressing CHO cells was less than that produced in human cells such as HeLa. CHO-HVEM12 cells infected with HSV-1(KOS) or HSV-1(F) produced 10,000-fold or 100-fold more progeny virus, respectively, than did control CHO-C8 cells or CHO-K1 cells, but 10-fold to 100-fold less than did HeLa cells.

III. HVEM Polypeptides

In another aspect, the present invention provides an HVEM polypeptide of mammalian origin. An HVEM of the present invention is a polypeptide of about 283 amino acid residues. Preferably, an HVEM is a human HVEM. A human form of HVEM is shown in SEQ ID NO:2. Thus, human HVEM can be defined as a polypeptide of about 283 or less amino acid residues comprising the amino acid residue sequence of SEQ ID NO:2.

The present invention also contemplates amino acid residue sequences that are substantially duplicative of the sequences set forth herein such that those sequences demonstrate like biological activity to disclosed sequences. Such contemplated sequences include those sequences characterized by a minimal change in amino acid residue sequence or type (e.g., conservatively substituted sequences) which insubstantial change does not alter the basic nature and biological activity of HVEM.

It is well known in the art that modifications and changes can be made in the structure of a polypeptide without substantially altering the biological function of that peptide. For example, certain amino acids can be substituted for other amino acids in a given polypeptide without any appreciable loss of function. In making such changes, substitutions of like amino acid residues can be made on the basis of relative similarity of side-chain substituents, for example, their size, charge, hydrophobicity, hydrophilicity, and the like.

As detailed in United States Patent No. 4,554,101, incorporated herein by reference, the following hydrophilicity values have been assigned to amino acid residues: Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4). It is understood that an amino acid residue can be substituted for another having a similar hydrophilicity value (e.g., within a value of plus or minus 2.0) and still obtain a biologically equivalent polypeptide.

In a similar manner, substitutions can be made on the basis of similarity in hydropathic index. Each amino acid residue has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. Those hydropathic index values are: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5). In making a substitution based on the hydropathic index, a value of within plus or minus 2.0 is preferred.

Comparisons of the HVEM amino acid sequence with those in the protein databases failed to identify any proteins closely related to HVEM, but did identify similarity of cysteine-rich repeats in the ectodomain of HVEM to those of the known human and animal members of the TNF/NGF receptor family (Armitage, 1994). Pairwise alignments and comparisons of the HVEM sequence with that of each of the other human members of the receptor family revealed sequence identities ranging from 16.9 to 25.4 % and identities plus similarities ranging from 26.9 to 37.1 %. However, cysteine and other residues conserved within the cysteine-rich repeat regions of members of the family were also conserved in HVEM (residues marked with asterisks in FIG. 2). Clearly, HVEM is a new member of the TNF/NGF receptor family.

The present invention also contemplates polypeptides comprising one or more of the functional domains of HVEM as well as polynucleotides that encode those domains. By way of example, a hybrid gene encoding a hybrid protein comprising functional domains of HVEM is described below.

Hybrid and epitope-tagged versions of HVEM were made to verify expression of the protein and to isolate material for immunization and detection of antibodies. The first 185 amino acids of the HVEM open reading frame,

encompassing the three and a half cysteine-rich repeats of the ectodomain (FIG. 2), were fused in frame to the hinge, CH2 and CH3 domains of a cDNA encoding the rabbit IgG heavy chain. Cells transfected with this expression construct secreted a glycoprotein, designated HVEM:Fc, that bound to protein G columns. This glycoprotein was heterogeneous in size (about 50-65 kDa). Changes in size observed after treatment of HVEM:Fc with glycosidases was consistent with the presence of O-linked carbohydrate chains and of complex N-linked chains. The fact that HVEM:Fc was glycosylated and secreted indicates that the hydrophobic region between amino acids 23 and 38 of HVEM can function as a signal sequence even though the N-terminus does not strictly adhere to rules for cleavable signal sequences. Rabbits were immunized with purified HVEM:Fc to produce anti-HVEM antibodies, the properties of which are described below.

An epitope-tagged version of HVEM was also engineered, by fusing the HVEM open reading frame from amino acids 1 to 257 (FIG. 2) to an oligonucleotide encoding an epitope from influenza virus hemagglutinin (Flu epitope) followed by a stop codon. Transfection of CHO-K1 cells with a plasmid expressing HVEM-257Flu resulted in production of several Flu-tagged HVEM species ranging in apparent size from about 30 to 90 kDa, as assessed by Western blotting with an anti-Flu monoclonal antibody. It is not known whether the higher molecular weight species are dimers or trimers of HVEM-257Flu or have posttranslational modifications such as large amounts of carbohydrate. HVEM-257Flu, even though deleted for the last 26 amino acids of the HVEM cytoplasmic tail, was fully functional for mediating HSV-1 entry.

The demonstration that a new member of the TNF/NGF receptor family, HVEM, can mediate or enhance the entry of HSV identifies another important family of cell surface receptors utilized by viruses for gaining entry to cells. In some instances, specific cell surface receptors serve principally as receptors for the binding of virus to cells. For example, members of the picornavirus family such as rhinoviruses and polioviruses bind to ICAM-1 (Tomassini et al., 1989; Staunton et al., 1989; Greve et al., 1989) or the poliovirus receptor PVR (Mendelsohn et al., 1989), respectively, both of which are cell surface glycoproteins belonging to the immunoglobulin superfamily. In other instances, multiple cell surface receptors may be required, not only for the binding of virus, but also to mediate viral penetration. Adenoviruses bind to cells via fibers extending from the vertices of the icosahedral virions, through interactions that

have not yet been defined. Entry is then facilitated by interaction of a protein at the base of each fiber with cell surface integrins (Wickham et al., 1993). Human immunodeficiency virus binds to cells via interaction of gp120 with CD4 (Dalgliesh et al., 1984; Klatzmann et al., 1984; Maddon et al., 1988) but entry requires cofactor activity which, for some virus strains, can be provided by a member of the G protein-coupled receptor family (Feng et al., 1996). In the case of HSV-1, binding of virus is to cell surface GAGs and, as shown here, entry was largely dependent on expression of the cell surface protein, HVEM. It should be noted that both GAGs and a mediator of entry such as HVEM are required for HSV infection, inasmuch as cells lacking GAGs (Shieh et al., 1992; Banfield et al., 1995) or a mediator can be 100 to 1000 times more resistant to infection than cells expressing both.

The classification of HVEM as a member of the TNF/NGF receptor family is based on the prediction from nucleotide sequence analysis that it is a type I membrane glycoprotein with three and a half cysteine-rich repeats characteristic of members of the family. The ligands for members of this receptor family fall into two classes, namely (i) TNF and related molecules that are produced as type II membrane proteins and may bind the receptor as membrane-bound ligands or soluble C-terminal domains (Armitage, 1994; Cosman, 1994) and (ii) neurotrophins such as NGF (Maness et al., 1994).

For several members of the TNF/NGF receptor family, binding of ligand results in aggregation of receptors, followed by binding of the cytoplasmic tails of the receptors to cytoplasmic molecules including TRAFs. Due to receptor aggregation itself or the binding of cytoplasmic factors, specific signal transduction pathways are activated (Heller and Krönke, 1994; Rothe et al., 1994). Binding of cognate receptors by members of the TNF family can have a variety of effects, including induction of cell proliferation, differentiation, or apoptosis, depending on the particular receptor, ligand and other factors (Smith et al., 1994). Binding of NGF to its receptor (low-affinity receptor) can influence whether other receptor-ligand interactions lead to cell survival or apoptosis (Rabizadeh and Bredesen, 1994; Maness et al., 1994). Interestingly, another human herpesvirus, Epstein-Barr virus, expresses a membrane protein, LMP-1, that interacts through its cytoplasmic domain with TRAFs, to trigger signal transduction in the absence of ligand binding to a member of the TNF receptor family (Mosialos et al., 1995). This activity of LMP-1 is thought to be important for cell immortalization and not for viral entry.

The ligand for HVEM and consequences of the ligand-receptor interaction remain to be identified. If a component of the HSV virion can interact with HVEM as an agonist, triggering of signal transduction pathways by this interaction could be critical for the viral entry pathway. There is no obvious relationship between HSV-1 envelope proteins and members of the TNF/NGF ligand family, although the virion component need not interact with HVEM in the same manner as its natural ligand. In fact, the role of HVEM in HSV entry could be completely dissociable from its normal physiological role in signaling. It will be important to determine whether the cytoplasmic domain of HVEM is required for HSV entry. Certainly, deletion of the C-terminal 26 amino acids has no effect on HSV entry. The membrane-proximal 32 amino acids in the cytoplasmic tail could, however, contain all the determinants necessary for interaction with cytoplasmic factors important for signal transduction, although no obvious homologies with known interactive domains are evident. Moreover, interactions involving exclusively the HVEM ectodomain or membrane-spanning region could transmit signals by other means. It seems quite likely that interactions of one or more of the dozen membrane glycoproteins in the HSV envelope with various cell surface molecules could transmit several types of signals to the cell interior. If so, the challenge will be to define those transmissions that influence viral entry and those that may influence later stages in infection.

The stage of HSV entry at which HVEM operates can be limited to specific steps of the entry process. These are (i) the membrane fusion reaction that occurs subsequent to the binding of virus to cell surface GAGs or (ii) the release and activation of virion proteins, including VP16, from the newly penetrated tegument and their transport to the cell nucleus. This follows from our findings that virus binding to CHO-K1 cells occurs efficiently in the absence of HVEM, or in the presence of anti-HVEM antibody. Also, the ability of HVEM to enhance HSV entry can be detected in CHO-IEβ8 cells, which carry a reporter gene under control of an immediate-early HSV-1 promoter. In these cells, introduction of input viral VP16 into the cell nucleus is all that is required to induce β-galactosidase expression and score an entry event.

The mechanism by which HVEM mediates HSV entry is not known at present. HVEM may interact directly with one or more of the virion envelope glycoproteins to trigger membrane fusion. The finding that HVEM:Fc can inhibit HSV infection is consistent with direct interaction with a virion component(s).

Candidates for this or these virion components include the four viral envelope glycoproteins (gB, gD, gH and gL) that are known to be required for HSV-1 entry, but not for the binding of virus to cells (Sarmiento et al., 1979; Cai et al., 1988; Ligas and Johnson, 1988; Forrester et al., 1992; Roop et al., 1993).

The best candidate for the virion component that might interact with HVEM is gD. First, previous studies have shown that, prior to penetration, cell-bound alphaherpesviruses can be eluted with soluble GAGs. However, presence of gD in virions can lead to elution-resistant binding (Karger and Mettenleiter, 1993; Fuller and Lee, 1992). Second, co-expression of HVEM and gD in CHO cells leaves the cells resistant to HSV-1 entry, as if HVEM were not expressed (M. Warner, R. I. Montgomery and P. G. Spear, unpublished observations). Previous reports have documented the ability of cell-associated gD to interfere with HSV entry into various types of normally susceptible cells (Campadelli-Fiume et al., 1988; Johnson and Spear, 1989). One obvious mechanism for this gD-mediated interference is sequestration of a cell receptor by cell-associated gD so that it is not available to interact with virion-associated gD. This is analogous to one of the mechanisms by which retrovirus glycoproteins are thought to interfere with viral entry or superinfection (Weiss, 1993).

Other members of the alphaherpesvirus subfamily resemble HSV in at least some of their requirements for entry into cells (Spear, 1993). For example, where sufficient information is available, it has been shown that human and animal alphaherpesviruses alike bind to cells through interactions with cell surface GAGs, usually heparan sulfate. Most express homologs of gB, gD, and gH-gL that are required for viral entry. Can HVEM or other members of the TNF/NGF receptor family mediate the entry of other alphaherpesviruses? This issue has not yet been thoroughly studied. We have found, however, that HVEM does not mediate PRV entry. This finding was not surprising, given the knowledge that cells susceptible to PRV entry are not necessarily susceptible to HSV entry and vice versa. However, evidence exists for some overlap of entry receptors utilized by HSV and PRV (Lee and Fuller, 1993) and cells expressing PRV gD can be resistant to the entry of both HSV and PRV (Petrovskis et al., 1988). A possible explanation is the existence of cell surface receptors that can mediate either HSV or PRV entry, or both, as discussed further below.

In previous studies, it had been proposed that a tyrosine kinase receptor for basic fibroblast growth factor (Kaner et al., 1990) or mannose-6-phosphate

receptors (Brunetti et al., 1995) might mediate the entry of HSV-1 into cells. Subsequent studies showed, however, that entry of HSV-1 was not dependent upon, nor influenced by, presence of these receptors (Shieh and Spear, 1991; Mirda et al., 1992; Muggeridge et al., 1992; Brunetti et al., 1995). For example, CHO-K1 cells transfected to express one of the receptors for basic fibroblast growth factor did not exhibit enhanced susceptibility to HSV entry (Shieh and Spear, 1991). In contrast, entry of HSV into CHO-K1 cells is significantly enhanced by transfection of the HVEM cDNA.

Several lines of evidence indicate that multiple cell surface molecules can mediate HSV entry by redundant mechanisms. First, although the cDNA for HVEM was isolated from a HeLa cell library, it seems likely that HVEM is not the principal mediator of HSV entry into HeLa cells. The anti-HVEM rabbit antibodies and HVEM:Fc had only marginal ability to block HSV-1 infection of HeLa cells. Second, CHO-K1 cells express some factor that can mediate the entry of certain HSV strains, especially HSV-2 strains. Third, continued screening of the HeLa cell cDNA library with other strains of HSV-1 indicates the presence of other genes that can mediate HSV entry. The fact that HVEM enhances the entry of all HSV-1 and HSV-2 strains tested, including recent clinical isolates, highlights its probable importance in HSV pathogenesis.

HVEM appears to be expressed in most human organs, especially lung, kidney and liver, based on the results of Northern blots. It remains to be determined which cell types express HVEM, and other mediators or co-receptors of HSV entry yet to be identified, in various organs and tissues. We predict that entry of HSV into certain differentiated cell types, such as those in mucosal epithelia, will be preferentially via one mediator and entry into other cell types, such as neurons, via a different mediator. In addition, the two serotypes of HSV or different strains of each serotype may use one entry mediator in preference to another, thus providing a molecular basis, in part, for different patterns of pathogenicity exhibited by different virus strains.

An HVEM polypeptide of the present invention has numerous uses. By way of example, such a polypeptide can be used in a screening assay for the identification of drugs or compounds that inhibit or augment the action of HVEM (e.g., agonist and antagonist to HSV entry into a cell). A screening assay for the identification of such compound, therefore, can be established whereby the ability of a compound to alter the action of HVEM can be determined by exposing cells

to HSV in the presence of a polypeptide of the present invention and varying amounts of compounds suspected of inhibiting the activity of HVEM.

The hybrid protein HVEM/Fc is used to immunize rabbits for the production of polyclonal antisera specific for the HVEM portion of the molecule. In addition the hybrid protein is used to screen for hybridomas secreting antibodies specific for the HVEM portion (the mice were immunized with HVEM-expressing CHO cells). The hybrid protein is used to determine whether a physical interaction between the hybrid protein and gD or other viral proteins can be detected. The hybrid protein also has use in screening expression cDNA libraries for natural ligands of HVEM and screening compounds for inhibitors of the interaction between HSV virions and HVEM.

Rabbit antiserum raised against HVEM:Fc was tested for the presence of antibodies specific for HVEM. CHO-K1 cells were transfected with a plasmid expressing HVEM-257Flu or control plasmids and cell extracts prepared. These were incubated with the rabbit antiserum or control pre-immune serum, followed by immunoprecipitation using protein A agarose and Western analyses for detection of the Flu epitope. The rabbit antiserum precipitated HVEM-257Flu, but not a Flu-tagged HSV-1 glycoprotein, whereas the pre-immune serum lacked specific precipitating activity. The anti-HVEM antibodies preferentially precipitated the higher molecular weight, and probably more highly processed, forms of HVEM-257Flu.

In addition, an HVEM polypeptide of the present invention can be used to produce antibodies that immunoreact specifically with HVEM. Means for producing antibodies are well known in the art. An antibody directed against HVEM can be a polyclonal or a monoclonal antibody.

Antibodies against HVEM can be prepared by immunizing an animal with an HVEM polypeptide of the present invention. Means for immunizing animals for the production of antibodies are well known in the art. By way of an example, a mammal can be injected with an inoculum that includes a polypeptide as described herein above. The polypeptide can be included in an inoculum alone or conjugated to a carrier protein such as keyhole limpet hemocyanin (KLH). The polypeptide can be suspended, as is well known in the art, in an adjuvant to enhance the immunogenicity of the polypeptide. Sera containing

immunologically active antibodies are then produced from the blood of such immunized animals using standard procedures well known in the art.

The identification of antibodies that immunoreact specifically with HVEM is made by exposing sera suspected of containing such antibodies to a polypeptide of the present invention to form a conjugate between antibodies and the polypeptide. The existence of the conjugate is then determined using standard procedures well known in the art.

An HVEM polypeptide of the present invention can also be used to prepare monoclonal antibodies against HVEM and used as a screening assay to identify such monoclonal antibodies. Monoclonal antibodies are produced from hybridomas prepared in accordance with standard techniques such as that described by Kohler et al. (Nature, 256:495, 1975). Briefly, a suitable mammal (e.g., BALB/c mouse) is immunized by injection with a polypeptide of the present invention. After a predetermined period of time, splenocytes are removed from the mouse and suspended in a cell culture medium. The splenocytes are then fused with an immortal cell line to form a hybridoma. The formed hybridomas are grown in cell culture and screened for their ability to produce a monoclonal antibody against HVEM. Screening of the cell culture medium is made with a polypeptide of the present invention.

IV. Method of Making HVEM

In another aspect, the present invention provides a process of making HVEM. In accordance with that process, a suitable host cell is transformed with a polynucleotide of the present invention. The transformed cell is maintained for a period of time sufficient for expression of the HVEM. The formed HVEM is then recovered.

Means for transforming host cells in a manner such that those cells produce recombinant polypeptides are well known in the art. Briefly, a polynucleotide that encodes the desired polypeptide is placed into an expression vector suitable for a given host cell. That vector can be a viral vector, phage or plasmid. In a preferred embodiment, a host cell used to produce HVEM is an eukaryotic host cell and an expression vector is an eukaryotic expression vector (i.e., a vector capable of directing expression in a eukaryotic cell). Such eukaryotic expression vectors are well known in the art.

In another embodiment, the host cell is a bacterial cell. An especially preferred bacterial cell is an *E. coli*. Thus, a preferred expression vector is a vector capable of directing expression in *E. coli*.

A polynucleotide of an expression vector of the present invention is preferably operatively associated or linked with an enhancer-promoter. A promoter is a region of a DNA molecule typically within about 100 nucleotide pairs in front of (upstream of) the point at which transcription begins. That region typically contains several types of DNA sequence elements that are located in similar relative positions in different genes. As used herein, the term "promoter" includes what is referred to in the art as an upstream promoter region or a promoter of a generalized RNA polymerase transcription unit.

Another type of transcription regulatory sequence element is an enhancer. An enhancer provides specificity of time, location and expression level for a particular encoding region (e.g., gene). A major function of an enhancer is to increase the level of transcription of a coding sequence in a cell that contains one or more transcription factors that bind to that enhancer. Unlike a promoter, an enhancer can function when located at variable distances from a transcription start site so long as the promoter is present.

As used herein, the phrase "enhancer-promoter" means a composite unit that contains both enhancer and promoter elements. An enhancer promoter is operatively linked to a coding sequence that encodes at least one gene product. As used herein, the phrase "operatively linked" or its grammatical equivalent means that a regulatory sequence element (e.g. an enhancer-promoter or transcription terminating region) is connected to a coding sequence in such a way that the transcription of that coding sequence is controlled and regulated by that enhancer-promoter. Means for operatively linking an enhancer-promoter to a coding sequence are well known in the art.

An enhancer-promoter used in an expression vector of the present invention can be any enhancer-promoter that drives expression in a host cell. By employing an enhancer-promoter with well known properties, the level of expression can be optimized. For example, selection of an enhancer-promoter that is active in specifically transformed cells permits tissue or cell specific expression of the desired product. Still further, selection of an enhancer-

promoter that is regulated in response to a specific physiological signal can permit inducible expression.

A coding sequence of an expression vector is operatively linked to a transcription terminating region. RNA polymerase transcribes an encoding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA). Enhancer-promoters and transcription-terminating regions are well known in the art. The selection of a particular enhancer-promoter or transcription-terminating region will depend, as is also well known in the art, on the cell to be transformed.

A clone of the human form of HVEM was identified by DNA sequence analysis as set forth above. This clone was used in all subsequent expression studies. HVEM was expressed in CHO-K1 cells under the control of a human cytomegalovirus promoter.

Expression vectors containing the encoding DNA sequence for all or a portion of human HVEM are designated pBEC580 (FIG. 6), pBEC10 (FIG. 7), and pBL58 (FIG. 8). Vectors were deposited, under the terms of the Budapest Treaty, on July 28, 1995 in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, and have been assigned ATCC Accession Nos: 97236 (pBEC580), 97235 (pBEC10), and 97237 (pBL58).

The present invention also contemplates a host cell transformed with a polynucleotide or expression vector of this invention. Means for transforming cells and polynucleotides and expression vectors used to transform host cells are set forth above. Preferably, the host cell is an eukaryotic host cell such as a mammalian cell or a prokaryotic cell such as an *E. coli*.

V. Pharmaceutical Compositions

The present invention also provides a pharmaceutical composition comprising a polypeptide or a polynucleotide of this invention and a physiologically acceptable diluent.

In a preferred embodiment, the present invention includes one or more antisense oligonucleotides or polypeptides, as set forth above, formulated into compositions together with one or more non-toxic physiologically tolerable or acceptable diluents, carriers, adjuvants or vehicles that are collectively referred to herein as diluents, for parenteral injection, for oral administration in solid or liquid form, for rectal or topical administration, or the like.

The compositions can be administered to humans and animals either orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, locally, or as a buccal or nasal spray.

Compositions suitable for parenteral administration can comprise physiologically acceptable sterile aqueous or non-aqueous solutions, dispersions, suspensions or emulsions and sterile powders for reconstitution into such sterile solutions or dispersions. Examples of suitable diluents include water, ethanol, polyols, suitable mixtures thereof, vegetable oils and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants.

Compositions can also contain adjuvants such as preserving, wetting, emulsifying, and dispensing agents. Prevention of the action of microorganisms can be insured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

Besides such inert diluents, the composition can also include sweetening, flavoring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonit, agar-agar and tragacanth, or mixtures of these substances, and the like.

The invention has been described in terms of preferred embodiments. One of ordinary skill in the art readily appreciates that changes and modifications

can be made to those embodiments without departing from the true scope of this invention.

Example 1: Cells and Viruses

Chinese hamster CHO-K1 cells, human HEp-2 cells, human HeLa cells, African green monkey (Vero) cells, and swine (ST) cells were obtained from the American Type Culture Collection. Human HT1080 cells were obtained from Dr. N. Bouck (Northwestern University). CHO-K1 cells were grown in Ham's F12 medium supplemented with 10% fetal bovine serum (FBS). Vero cells were grown in medium 199 supplemented with 5% FBS. All other cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS. Vero-gL cells, stably transfected with a plasmid expressing HSV-1 gL under control of a viral promoter were maintained in DMEM supplemented with 10% FBS and Geneticin at 400 μg/ml. SW78 cells, which were derived from Vero cells stably transfected with a plasmid expressing PRV gH (Klupp et al., 1994), were obtained from T. C. Mettenleiter (Friedrich-Loeffler-Institutes, Germany) and maintained in the same medium containing Geneticin at 700 μg/ml. All media components were purchased from GIBCO Laboratories except FBS, which was obtained from Sigma Chemicals.

The CHO-IEβ8 cell line was isolated after transfection of CHO-K1 cells with pMLP01, which carries a puromycin resistance gene and the E. coli *lacZ* gene under control of the HSV-1 ICP4 promoter. This plasmid was generated by insertion of the appropriate BamHI fragment from pON105 (Ho and Mocarski, 1988) into the single BamHI site of pPUR (Clontech). Stable transfectants, which were resistant to puromycin and expressed β-galactosidase only upon infection of the cells with HSV, were isolated and cloned by limiting dilution. Induction of β-galactosidase expression in these cells requires entry of virus into the cell and delivery of the tegument protein VP16 to the cell nucleus, but does not require viral gene expression from the input viral genome.

Virus strains used included several standard laboratory strains and well-characterized clinical isolates: HSV-1(KOS); HSV-1(HFEM); HSV-1(Patton); HSV-1(F) (Ejercito et al., 1968); HSV-1(SC16); HSV-1(17) (McGeoch et al., 1988) and HSV-2(333). Two mutants .ains recognized by their syncytial phenotypes were also included: HSV-1(KOS)804 (Little and Schaffer, 1981); HSV-1(MP) (Hoggan and Roizman, 1959). These strains were propagated by low multiplicity passage on HEp-2 cells and were titrated on Vero cells.

HSV-1(KOS)gL86 is a gL-negative mutant in which the E. coli *lacZ* gene under control of the CMV promoter replaces part of the gL open reading frame. This mutant was propagated and titered on Vero-gL cells to complement deletion of the gL gene from the viral genome. A similar PRV mutant, in which the gH gene was interrupted by insertion of the *lacZ* gene (Klupp et al., 1994), was obtained from T. C. Mettenleiter and propagated and titered on gH-expressing SW78 cells. Mutant viruses obtained from these complementing cell lines were fully infectious for, and expressed β-galactosidase in, non-complementing cells but were able to undergo only one round of replication on non-complementing cells.

Example 2: Infectivity Assays

Infectivity assays were performed with the β-galactosidase-expressing viruses, HSV-1(KOS)gL86 or the gH-negative PRV, on a variety of cell lines or with a variety of virus strains on the cell line CHO-IEβ8, which expresses β-galactosidase only after entry of HSV-1 or HSV-2. Cells were plated in 96-well tissue culture dishes (2-4 x 104 cells/well) at least 16 hours prior to infection. The cells were rinsed with phosphate-buffered saline containing glucose and 1% calf serum (PBS-G-CS) and then inoculated with various concentrations of virus in 50 µl of PBS-G-CS. After incubation of the infected cells at 37°C for 6 hours, the cells were washed with PBS and solubilized in PBS containing 0.5% NP40 and the β-galactosidase substrate, o-nitrophenyl β-D-glucopyranoside, at 3 mg/ml. Production of the colored reaction product was quantitated several times over an 18 hr period by determining the optical density at 410 mm, using a Dynatech ELISA reader or a Spectromax 250. Alternatively, cells were plated in 6-well tissue culture dishes and exposed to virus to determine the numbers of cells that became infected, as assessed by use of the β-galactosidase substrate X-gal(GIBCO Laboratories), which yields an insoluble blue reaction product. At 6 hours after infection, the cells were washed with PBS, fixed in PBS containing 2% formaldehyde and 0.2% gluteraldehyde, permeabilized in 2 mM MgCl2 containing 0.01% deoxycholate and 0.02% NP40 and incubated with buffered X-gal at 0.5 mg/ml. Infectivity assays done to test the ability of antibodies to protect cells from infection were performed essentially as described except that rabbit serum diluted in PBS was added to the cells (50 µl per well) 30 minutes prior to the addition of virus (10 µl per well). After 2 hours of incubation at 37°C with the serum-virus mixtures, the cells were treated with 100 mM citrate buffer, pH 3.0, for 1 minute to inactivate virus that had not yet penetrated the cells (Huang and Wagner, 1964; Highlander et al., 1987). The cells were then washed with PBS and incubated in medium 199 plus 1% FBS for 4 hours prior to

solubilization and quantitation of β-galactosidase activity. For tests of the ability of HVEM:Fc to neutralize viral infectivity, the assay was the same except that various dilutions of virus were mixed with HVEM:Fc or rabbit IgG at different concentrations and incubated for 30 minutes at 37°C prior to addition of the mixtures to washed cell monolayers.

The binding of virus to cells was quantitated as described previously (Shieh et al., 1992) using 3H-thymidine-labeled purified HSV-1(KOS).

Example 3: Isolation and Sequencing of the HVEM cDNA

A unidirectional HeLa cell cDNA expression library cloned into pcDNA1 in E. coli (InVitrogen) was plated onto 100 150-mm LB plates containing appropriate drugs at about 1.5 x 105 bacteria per plate. The colonies on each plate were pooled by scraping and frozen as 15% glycerol stocks. Samples of each stock were combined into groups of 10 and grown to stationary phase in 250 ml of broth. Plasmids prepared from each culture were transfected into CHO-K1 cells using LipofectAMINE (GIBCO Laboratories) as described by the manufacturer (1.5 μg of plasmid and 5 μl of LipofectAMINE per 35 mm culture). For controls, the cells were transfected with pMN84, a plasmid containing the E. coli *lacZ* gene under control of the CMV promoter or were incubated with LipofectAMINE alone. At 30 hours after transfection and medium changes as recommended, the cells were washed with PBS-G-CS and inoculated with HSV-1(KOS)gL86 at about 300 PFU per cell. Control cultures transfected with pMN84 were not exposed to virus. At 6 hours after exposure to virus the cells were then fixed and stained with X-gal as described above. Transfection efficiencies ranged from 30-55% of cells based on expression of β-galactosidase from the positive control plasmid pMN84. In the first round of screening the cDNA library, one group of 10 bacterial stocks from the library converted about 20-30 cells in the monolayer to susceptibility to HSV-1(KOS)gL86 infection. The frequency of conversion to susceptibility was about 10 times higher for one of the 10 stocks in this group. This stock was divided again into 100 pools and, by an iterative process, two bacterial clones were obtained that yielded plasmids capable of converting transfected CHO-K1 cells to susceptibility to HSV-1(KOS)gL86 infection. These plasmids were designated pBEC580 and pBEC748.

Both strands of the cDNA insert of pBEC580 (FIG. 6), were sequenced using the dideoxynucleotide chain termination method (Sequenase Kit Version

2.0, Amersham) and T7 and Sp6 primers, as well as other sense and anti-sense primers generated as sequence was obtained from the insert. All primers were obtained from the Northwestern University Biotechnology Center. Single-strand sequencing of pBEC748 revealed the cDNA insert to be identical to that of pBEC580.

Genomic DNAs were extracted from cell lysates (Hirt, 1967), digested with BamHI and the fragments separated by electrophoresis on 0.8% agarose. After ethidium bromide staining, the DNA fragments were transferred to a Duralon nylon membrane and cross-liked to the membrane using ultraviolet light. Randomly primed probes were generated using EcoRI or PvuII fragments of the HVEM insert as template and digoxigen-labeled nucleotides (Genius Kit; Boehringer Mannheim) or 32P-labeled nucleotides for synthesis. Pre-hybridization was for 2 hours in 50% formamide, 6X SSC buffer (hybridization buffer). Hybridization was at 42°C for 24 hours in hybridization buffer. Washing was with 2X SSC containing 0.1% SDS followed by 2 cycles of incubation for 15 minutes at 65°C with 0.2X SSC containing 0.1% SDS and a final wash in the same solution. The blot was exposed to X-ray film at -70°C (for 32P-labeled probes) or processed for digoxigen detection by chemiluminescence as described by the manufacturer (Boehringer Mannheim), utilizing the alkaline phosphatase substrate CDP-STAR™ (Tropix) with visualization by exposure to Amersham Hyperfilm-MP.

Northern analysis was performed using a commercial blot of polyadenylated RNAs extracted from various human tissues, which included heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas (Clontech). A randomly primed 32P-labeled probe homologous to the HVEM PvuII fragment was produced as described above. Prehybridization and hybridization of the blot were performed as described above at 42°C and washes with 0.2X SSC containing 0.1% SDS were at 50°C. The blot was exposed to X-ray film, with intensifying screen, at -70°C.

Example 4: Plasmids and Stably Transfected Cell Lines

A plasmid carrying the HVEM insert and a neomycin-resistance gene, designated pBEC10 (FIG. 7), was generated by excision of the HVEM insert from pBEC580 with HindIII and XhoI followed by its insertion between the HindIII and XhoI sites in the polylinker of pcDNA3. A plasmid expressing a truncated epitope-tagged version of HVEM (HVEM-257Flu) was generated in

several steps and designated pBEC14. This plasmid contains the HindIII to SfiI fragment of the HVEM insert from pBEC580, which was modified by deletion of sequences in the upstream polylinker and 5' untranslated region, between the BamHI and BstYI sites. This modified HindIII to SfiI fragment was inserted between HindIII and EcoRI sites of pMN104 (blunt-end ligation between the SfiI and EcoRI sites after Klenow treatment). pMN104 contains an oligonucleotide (TACCCATACGATGTTCCAGATTACGCTAGCTTGTAA), (SEQ ID NO:3) inserted between the EcoRI and XbaI sites of pcDNA3, that encodes 11 amino acids (EFYPYDVPDYASL) (SEQ ID NO:4) plus a stop codon, including a 9-amino acid epitope (underlined) from the influenza virus hemagglutinin (Wilson et al., 1984). A plasmid expressing a hybrid form of HVEM, linking the HVEM ectodomain to the hinge, CH2 and CH3 region of the rabbit IgG heavy chain, was generated in several steps and designated pBL58. This plasmid contains several elements linked in the following order: a cytomegalovirus promoter from pcDNAneo (SpeI to HindIII); a portion of the polylinker from pGEM3 (HindIII to XbaI); a portion of the HVEM insert from pBEC580 (NheI site located about 37 nucleotides upstream of the start codon to a PvuII site just downstream of the last Cys residue in the ectodomain); a fragment of the rabbit IgG heavy chain cDNA from plasmid 3-4 (obtained from K. Knight at Loyola University Medical Center) including an EcoRI site added by PCR 5' to the rabbit sequence ACAAGACCGTTGC (SEQ ID NO:5) and extending to a PstI site downstream of the reading frame (after cleavage with EcoRI, the filled-in site was blunt-end ligated to the PvuII end of the HVEM fragment); pGEM4 plasmid sequences from the HindIII site to the NheI site (the PstI end of the rabbit sequence was blunt-end ligated to the HindIII site and the NheI site was sticky-end ligated to the SpeI site of the CMV promoter fragment.

To produce cell lines stably expressing HVEM, CHO-K1 cells and ST cells were transfected with pBEC10, as described above, and replated after 24 hours in selective medium containing Geneticin at 500 µg/ml for CHO-K1 cells and at 800 µg/ml for ST cells. After 2 weeks the cells surviving selection were detached and cloned by limiting dilution in 96-well dishes using the same selective conditions. Cell clones were tested for their ability to be infected by HSV-1(KOS)gL86, using X-gal substrate, and positive clones were subcloned and expanded. Control cell lines were produced by transfecting CHO-K1 and ST cells with pcDNA3 and isolating Geneticin-resistant clones as described above.

Example 5: HVEM:Fc production, characterization and use as immunogen

For production of HVEM:Fc, CHO-K1 cells were transfected with pBL58 using LipofectAMINE and maintained in F12 medium supplemented with 3% FBS. Medium was harvested from the cultures every 24 hours for 3 days and HVEM:Fc was purified from the medium by Protein G-Sepharose chromatography. Polyclonal antibodies were raised against HVEM:Fc at Pocono Farm, Inc., by subcutaneous injection of purified protein mixed with Hunter's TiterMax adjuvant into a New Zealand white female rabbit. To characterize the carbohydrate modifications of HVEM:Fc, samples were denatured by boiling for 5 minutes in 0.5% SDS, 1% β-mercaptoethanol and then incubated overnight at 37°C with endo F (200 mU, 1% NP-40, 50 mM sodium phosphate buffer, pH 7.5) or endo H (1 mU, 50 mM sodium citrate buffer, pH 5.5) or without added enzyme (1% NP-40, 50 mM sodium phosphate buffer, pH 7.5). Alternatively, samples were first digested overnight at 37°C with neuraminidase (4 mU, 50 mM sodium citrate buffer, pH 4.5), then denatured as above and incubated overnight at 37°C with endo F (200 mU) and O-glycosidase (0.5 mU) in 1% NP-40, 50 mM sodium phosphate buffer, pH 7.5. Control and glycosidase-treated samples were subjected to SDS-PAGE on a 12% gel and then transferred to nitrocellulose for Western blotting. The blot was probed for the rabbit IgG Fc domain of the HVEM:Fc hybrid protein by use of a mixture of anti-rabbit IgG peroxidase conjugates (GibcoBRL 9814SA and Sigma A6667) at concentrations of 1:1000 in BLOTTO (10 mM Tris, pH 7.4, 150 mM NaCl, 5% powdered milk, 0.05% tween-20) followed by chemiluminescent detection with ECL reagent and Hyperfilm-MP (Amersham).

Antigens used were lysates from CHO-K1 cells transfected with pBEC14 to express HVEM-257Flu or with two control plasmids (pMN91 which expresses an epitope-tagged truncated version of an HSV-1 glycoprotein [gL(94)Flu] or pcDNA3 which expresses no epitope-tagged protein). The transfected cells were harvested by scraping, washed with PBS and lysed on ice with 1% Triton X-100 in 150 mM NaCl, 20 mM Tris-HCl, pH 8.0, containing a mixture of protease inhibitors (aprotinin at 2 μg/ml; leupeptin at 2 μg/ml; pepstatin A at 1 μg/ml; 5 mM phenylmethylsulfonyl fluoride). The cell lysates were cleared by centrifugation and used immediately for immunoprecipitation or were immediately prepared for SDS-PAGE. For immunoprecipitation, samples of the cell lysates were mixed on ice with rabbit pre-immune or immune serum (10 μl per 200 μl of lysate) and, after 1 hr, a suspension of Protein A-agarose

(Boehringer Mannheim) was added (100 µl of a 10% suspension) for an additional 2 hours, with rotation, at 4oC. The Protein A-agarose was collected by centrifugation, washed and the bound proteins eluted for separation by SDS-PAGE. Samples of the cell lysates and the immunoprecipitates obtained from the cell lysates were subjected to electrophoresis on 13% polyacrylamide gels and then transferred to nitrocellulose membranes. The membranes were incubated in BLOTTO for I hr of blocking and then with mouse ascites fluid containing the anti-hemagglutinin antibody 12CA5 (Wilson et al., 1984) diluted 1:5000 in BLOTTO. The second antibody was horseradish peroxidase-coupled goat anti-mouse IgG (Boehringer Mannheim) diluted 1:10,000. Detection of second antibody bound to the blots was by incubation in ECL reagent and exposure to Amersham Hyperfilm-MP (Amersham).

Autoradiograms were scanned using a Bio-Rad GS-670 Imaging Densitometer. Slides obtained by light microscopy were scanned using a Polaroid Sprint scanner. Prints were produced using Adobe Photoshop and Canvas™ 3.5 and printed on a Tektronic Phase II_{SDX} Printer. Facilities used were those of the Northwestern digital darkroom.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Northwestern University

(ii) TITLE OF INVENTION: Herpes Virus Entry Mediator

(iii) NUMBER OF SEQUENCES: 5

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1724 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 294..1145

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 294..1142

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CCCCCTGCTG CCCACTCTCC TGCTGCTCGG GTTCTGAGGC ACAGCTTGTC ACACCGAGGC     180

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CAG CGG AAA AGA CAG GAG GCA GAA GGT GAG GCC ACA GTC ATT GAG GCC     1064
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 283 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gln Met Cys Asp Pro Ala Met Gly Leu Arg Ala Thr Arg Asn Cys Ser
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Arg Thr Glu Asn Ala Val Cys Gly Cys Ser Pro Gly His Phe Cys Ile
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Val Gln Asp Gly Asp His Cys Ala Ala Cys Arg Arg Tyr Ala Thr Ser
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145                 150                 155                 160

Leu Cys Gln Asn Cys Pro Pro Gly Thr Phe Ser Pro Asn Gly Thr Leu
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225                 230                 235                 240

Val Gln Arg Lys Arg Gln Glu Ala Glu Gly Glu Ala Thr Val Ile Glu
                245                 250                 255

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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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(2) INFORMATION FOR SEQ ID NO:5:

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(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACAAGACCGT TGC 13

WHAT IS CLAIMED IS:

1. An isolated and purified polypeptide of about 283 amino acid residues comprising the amino acid residue sequence of SEQ ID NO:2.

2. A recombinant human HVEM.

3. A process of detecting an antibody against HVEM in a biological sample comprising adding the polypeptide of claim 1 to the sample, maintaining the sample for a period of time sufficient for formation of a conjugate between the antibody and the polypeptide and detecting the presence of the conjugate and thereby the antibody.

4. An isolated and purified polynucleotide comprising a nucleotide sequence consisting essentially of a nucleotide sequence selected from the group consisting of: a) the sequence of SEQ ID NO:1 from nucleotide position 294 to about nucleotide position 1142; b) sequences that are complementary to the sequence of (a); c) sequences that, on expression, encode a polypeptide encoded by the sequence of (a).

5. The polynucleotide of claim 4 that is a DNA molecule.

6. The polynucleotide of claim 5 wherein the nucleotide sequence is SEQ ID NO:1.

7. The polynucleotide of claim 4 that is an RNA molecule.

8. An expression vector comprising the DNA molecule of claim 5.

9. The expression vector of claim 8 further comprising an enhancer-promoter operatively linked to the polynucleotide.

10. The expression vector of claim 8 wherein the polynucleotide has the nucleotide sequence of SEQ ID NO:1 from nucleotide position 294 to about nucleotide position 1142.

11. An oligonucleotide of from about 15 to about 50 nucleotides containing a nucleotide sequence of at least 15 nucleotides that is identical or complementary to a contiguous sequence of the polynucleotide of claim 4.

12. The oligonucleotide of claim 11 that is an antisense oligonucleotide.

13. A host cell transformed with the expression vector of claim 8.

14. The transformed host cell of claim 13 that is a mammalian cell.

15. The transformed host cell of claim 13 that is a bacterial cell.

16. The transformed host cell of claim 14 wherein the mammalian cell is an ovarian cell.

17. The transformed host cell of claim 16 wherein the ovarian cell is designated CHO-A3, CHO-A12, CHO-B3, CHO-B9, or CHO-B11.

18. A process of making HVEM comprising transforming a host cell with the expression vector of claim 8, maintaining the transformed cell for a period of time sufficient for expression of the HVEM and recovering the HVEM.

19. The process of claim 18 wherein the host cell is an eukaryotic host cell.

20. The process of claim 19 wherein the host cell is a mammalian cell.

21. The process of claim 20 wherein the mammalian cell is an ovarian cell.

22. The process of claim 18 wherein the HVEM is human HVEM.

23. The process of claim 18 wherein the polynucleotide has the nucleotide sequence of SEQ ID NO:1 from nucleotide position 294 to about nucleotide position 1142.

24. HVEM made by the process of claim 18.

25. A pharmaceutical composition comprising the oligonucleotide of claim 12 and a physiological acceptable diluent.

26. A pharmaceutical composition comprising the polypeptide of claim 1 and a physiologically acceptable diluent.

27. A plasmid selected from the group consisting of pBEC10, pBEC580, and pBL58.

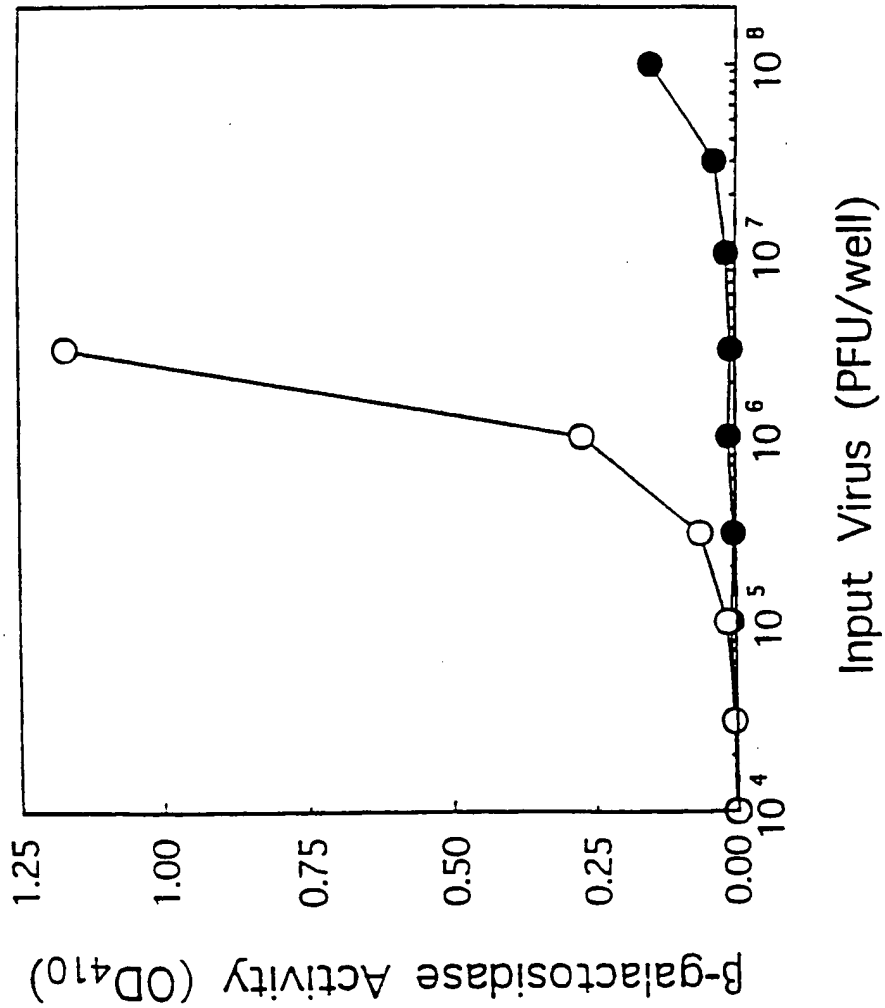

FIG. 1

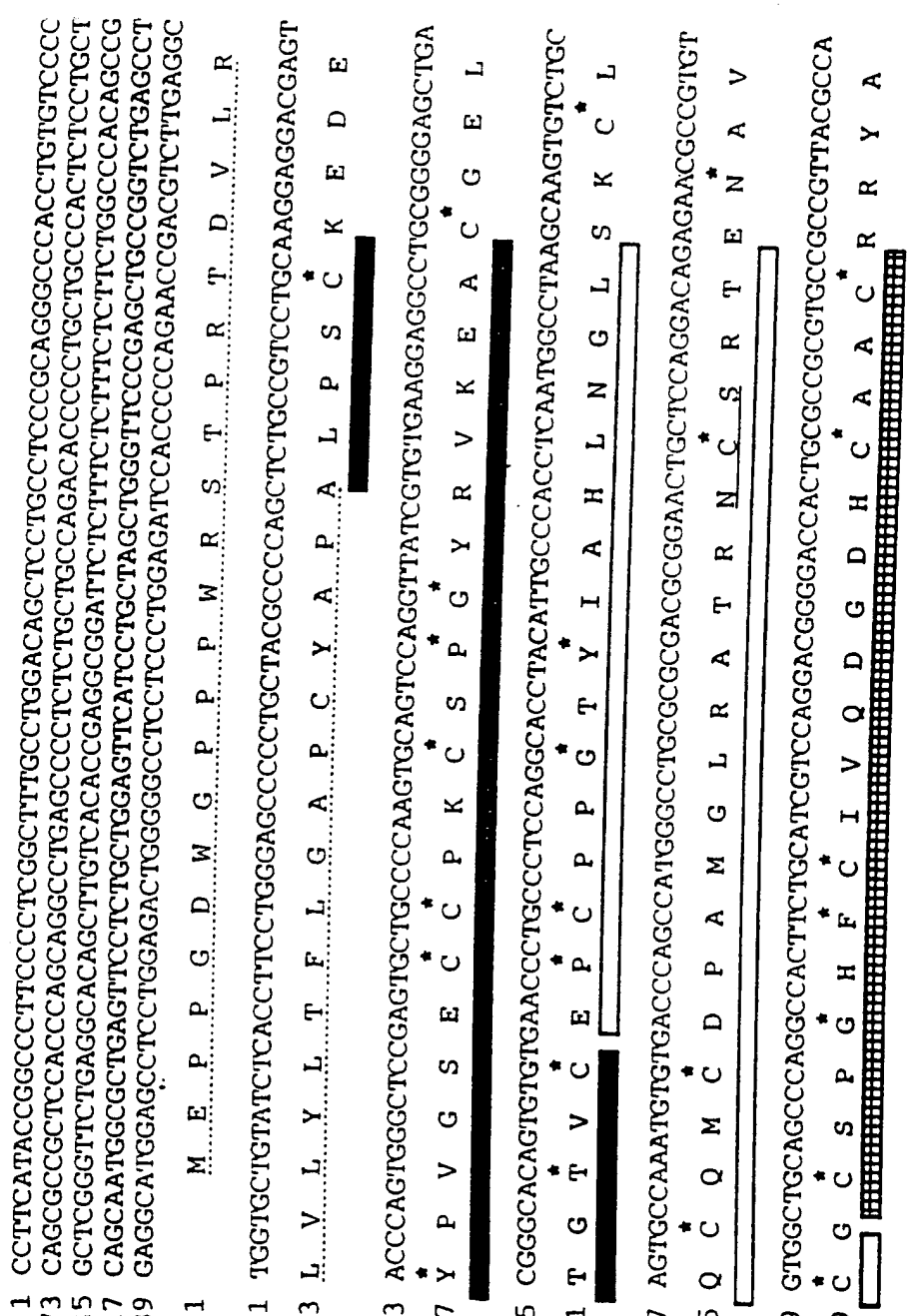

FIG. 2

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721  CCTCCAGCCCCGGGCCAGAGGGTGCAGAAGGGAGGCACCGAGAGTCAGGACACCCTGTGTCAGAACTGCCCCC
143  T  S  S  P  G  Q  R  V  Q  K  G  G  G  T  E  S  Q  D  T  L  C  Q  N  C  P
793  CGGGGACCTTCTCTCCCAATGGGACCCTGGAGGAATGTCAGCACCAGACCAAGTGCAGCTGGCTGGTGACGA
167  P  G  T  F  S  P  N  G  T  L  E  E  C  Q  H  Q  T  K  C  S  W  L  V  T
865  AGGCCGGAGCTGGGACCAGCAGCTCCCACTGGGTATGGTGGTTTCTCTCAGGGAGCCTCGTCATCGTCATTG
191  K  A  G  G  A  G  T  S  S  S  H  W  V  W  W  F  L  S  G  S  L  V  I  V  I
937  TTTGCTCCACAGTTGGCCTAATCATATGTGTGAAAAGAAGAAGCCAAGGGGTGATGTAGTCAAGGTGATCG
215  V  C  S  T  V  G  L  I  I  C  V  K  R  R  K  P  R  G  D  V  V  K  V  I
1009 TCTCCGTCCAGCGGAAAAGACAGGAGGCAGAAGGTGAGGCCACAGTCATTGAGGCCCTGCAGGCCCCTCCGG
239  V  S  V  Q  R  K  R  Q  E  A  E  G  E  A  T  V  I  E  A  L  Q  A  P  P
1081 ACGTCACCACGGTGGCCGTGGAGGAGACAATACCCTCATTCACGGGGAGGAGCCCAAACCACTGACCCACAG
263  D  V  T  T  V  A  V  E  E  T  I  P  S  F  T  G  R  S  P  N  H  -
1153 ACTCTGCACCCCGACGCCAGAGATACCTGGAGCGACGGCTGCTGAAAGAGGCTGTGTCCACCTGGCGAAACCAC
1225 CGGAGCCCGGAGGCTTGGGGGCTCCGCCCTGGGCTGGCTTCCGTCTCCTCCAGTGGAGGGAGAGGTGGGGCC
1297 CCTGCTGGGGTAGAGCTGGGGACGCCACGTGCCATTCCCATGGGCCAGTGAGGGCCTGGGGCCTCTGTTCTG
1369 CTGTGGCCTGAGCTCCCCAGAGTCCTGAGGAGGAGCGCCAGTTGCCCCTCGCTCACAGACCACACACCCAGC
1441 CCTCCTGGGCCAGCCCAGAGGGCCCTTCAGACCCCCAGCTGTCTGCGCGTCTGACTCTTGTGGCCTCAGCAGG
1513 ACAGGCCCCGGGCACTGCCTCACAGCCAAGGCTGGACTGGGTTGGCTGCAGTGTGGTGTTTAGTGGATACCA
1585 CATCGGAAGTGATTTTCTAAATTGGATTTGAATTCCGGTCCTGTCTTCTATTTGTCATGAAACAGTGTATTT
1657 GGGGAGATGCTGTGGGAGGATGTAAATATCTTGTTTCTCCTCAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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FIG. 2 (continued)

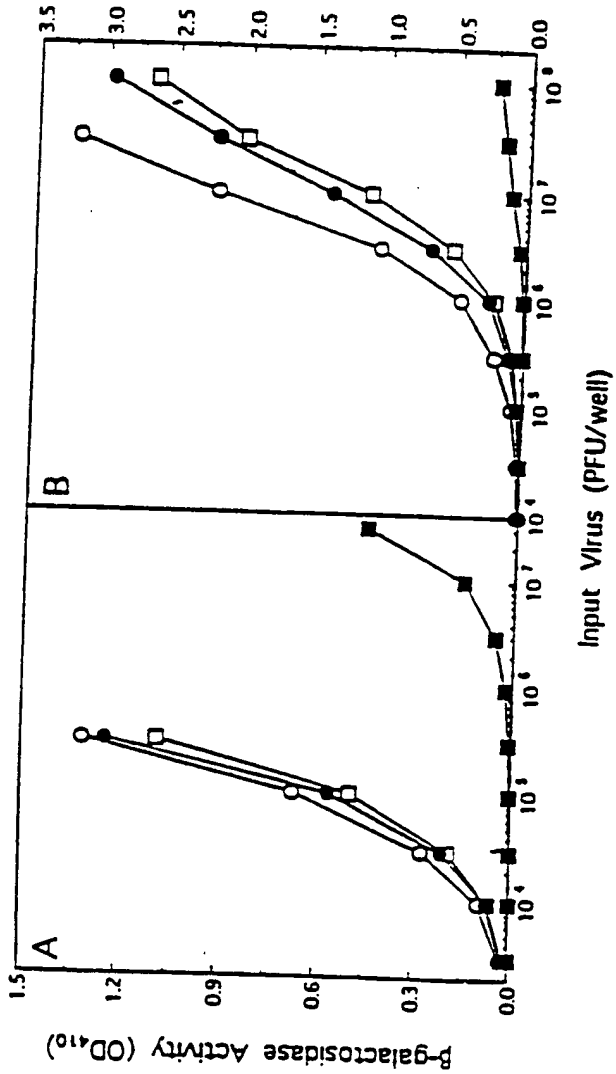

FIG. 3

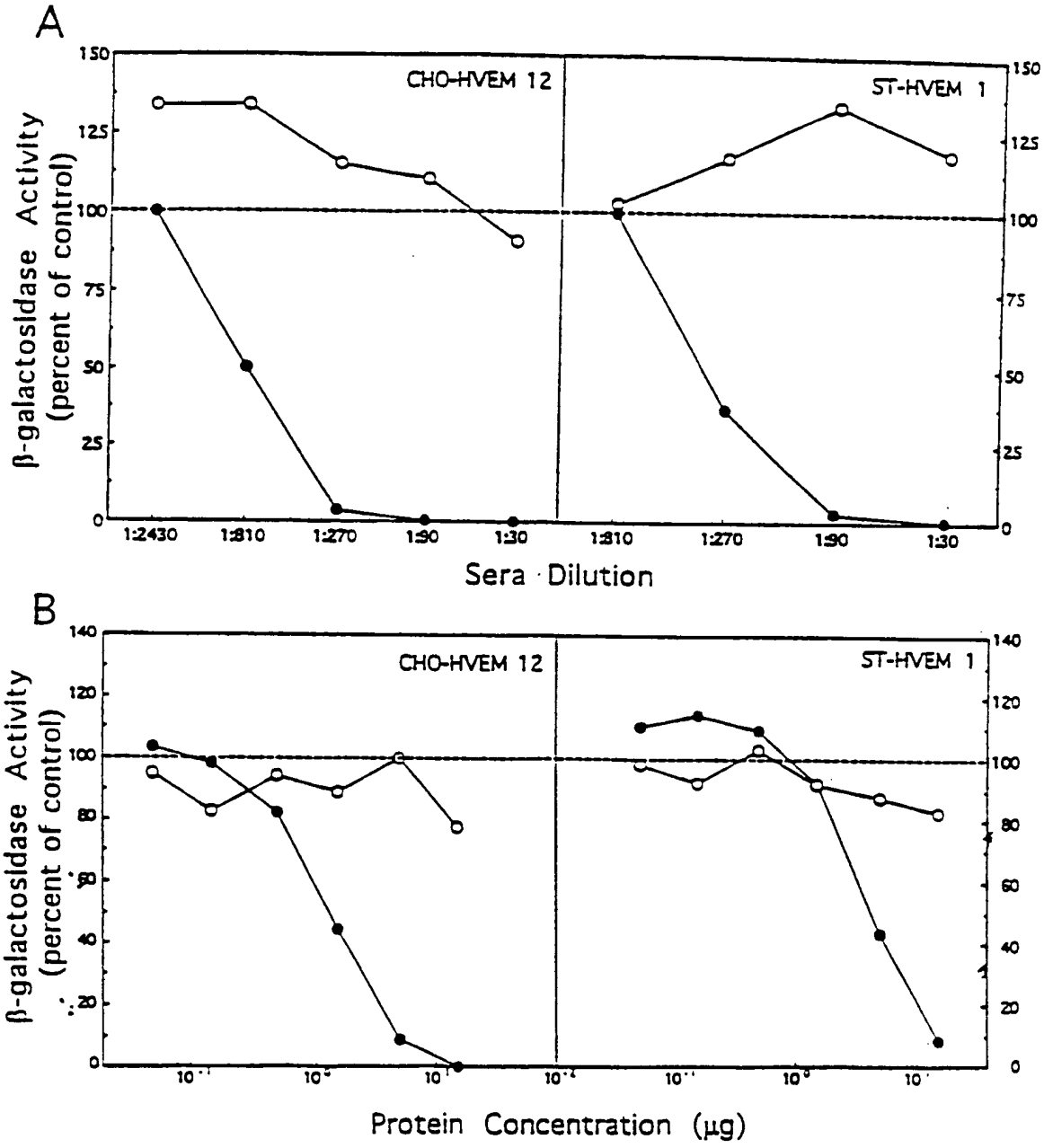

FIG. 4

SUBSTITUTE SHEET (RULE 26)

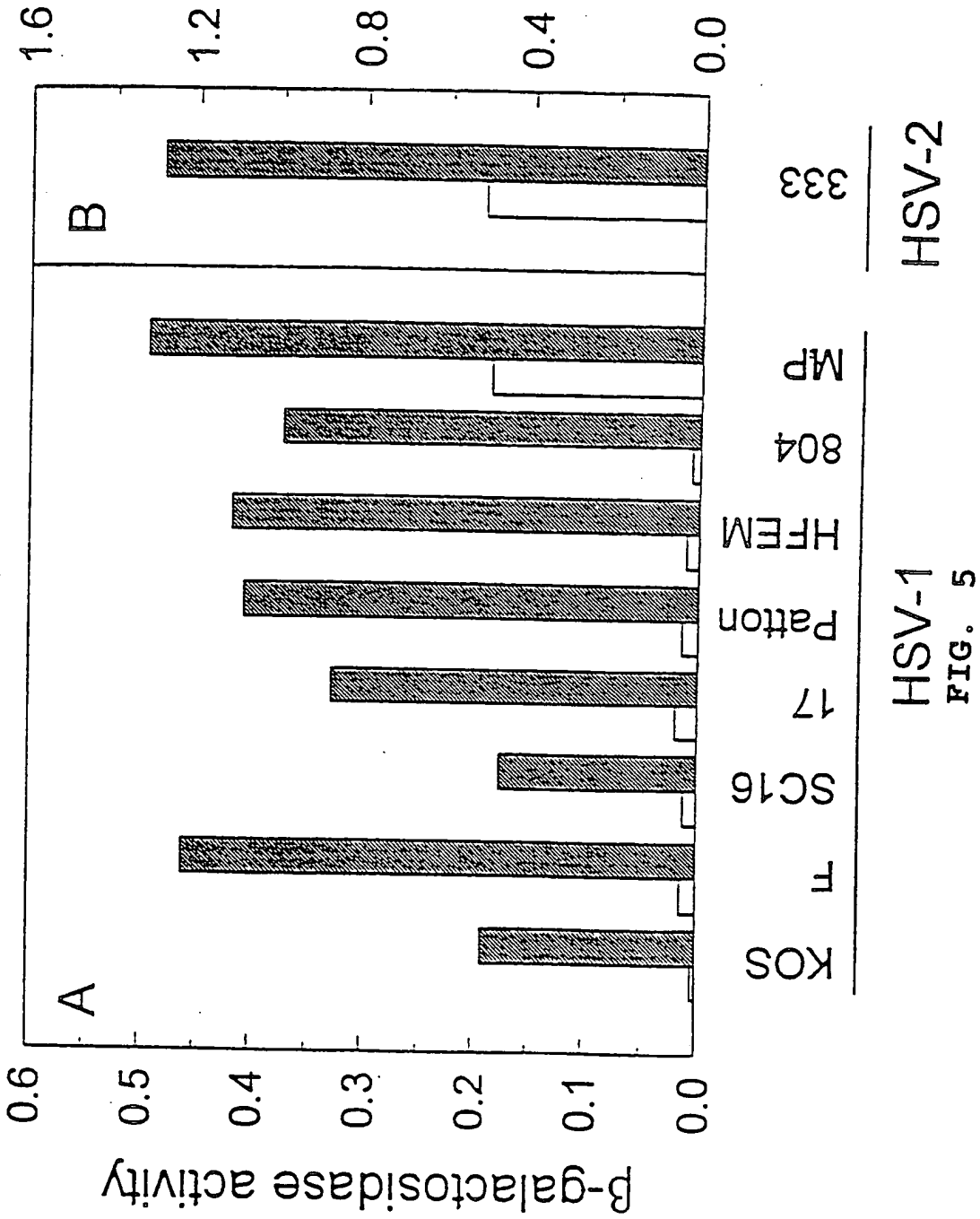

FIG. 5

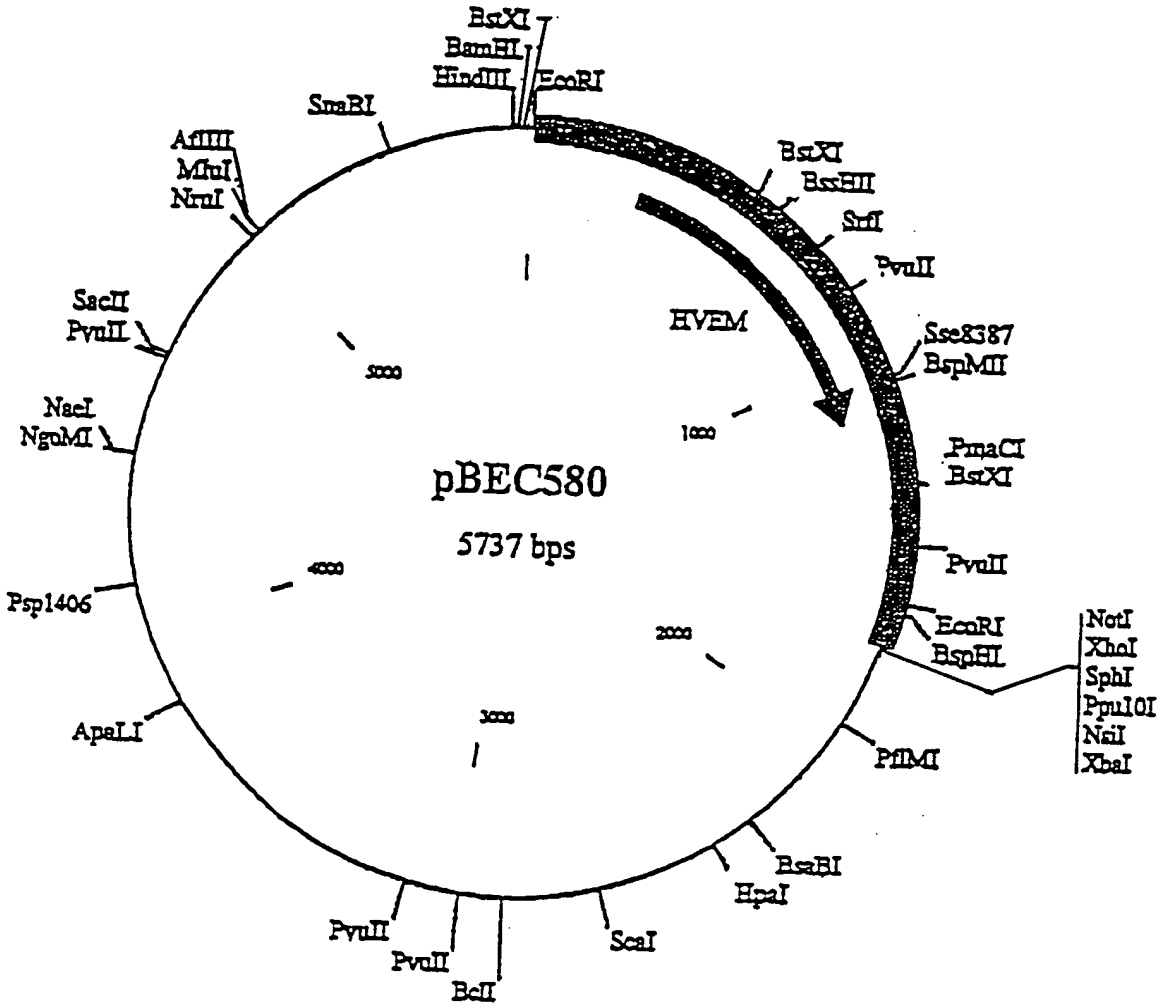

FIG. 6

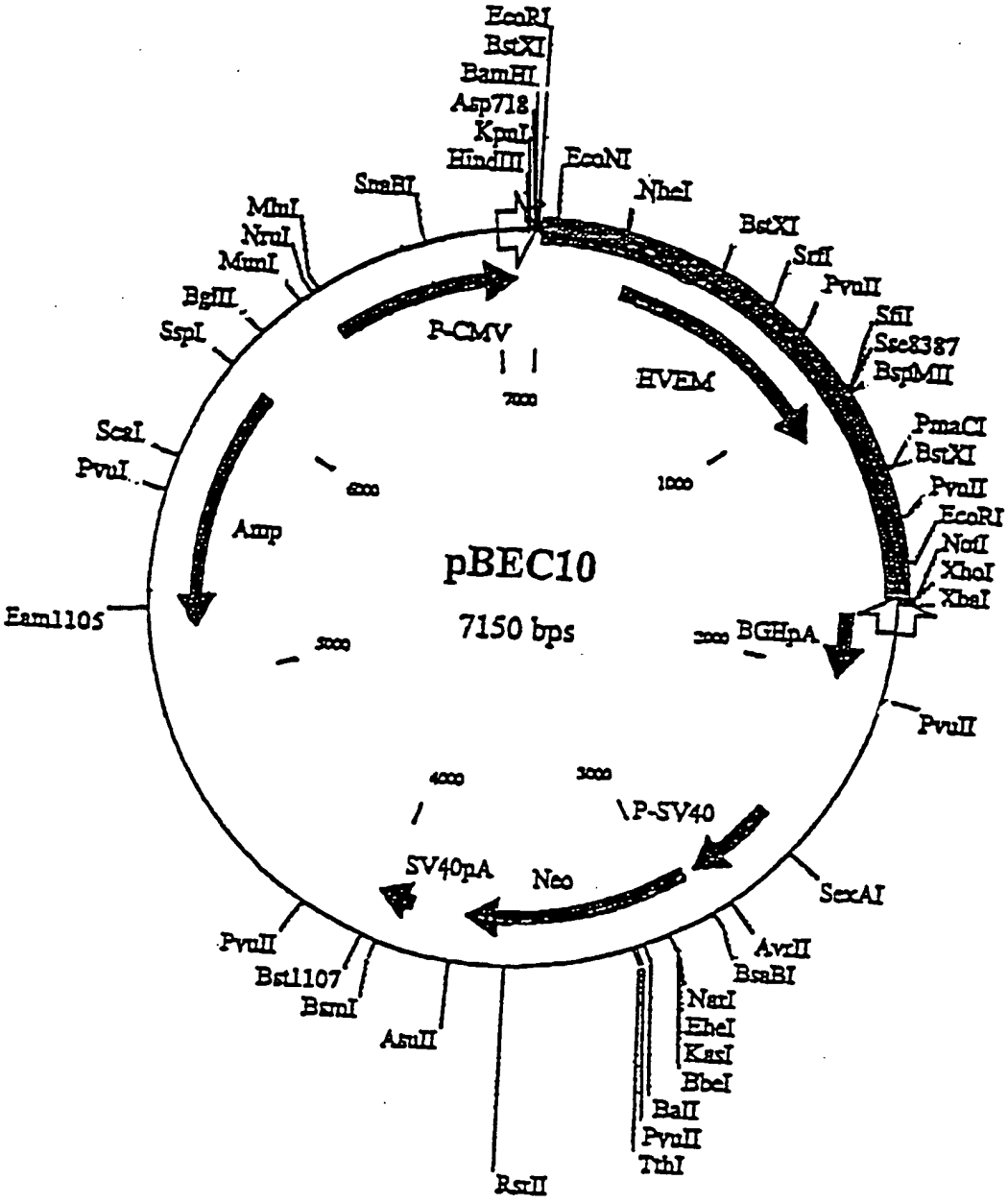

FIG. 7

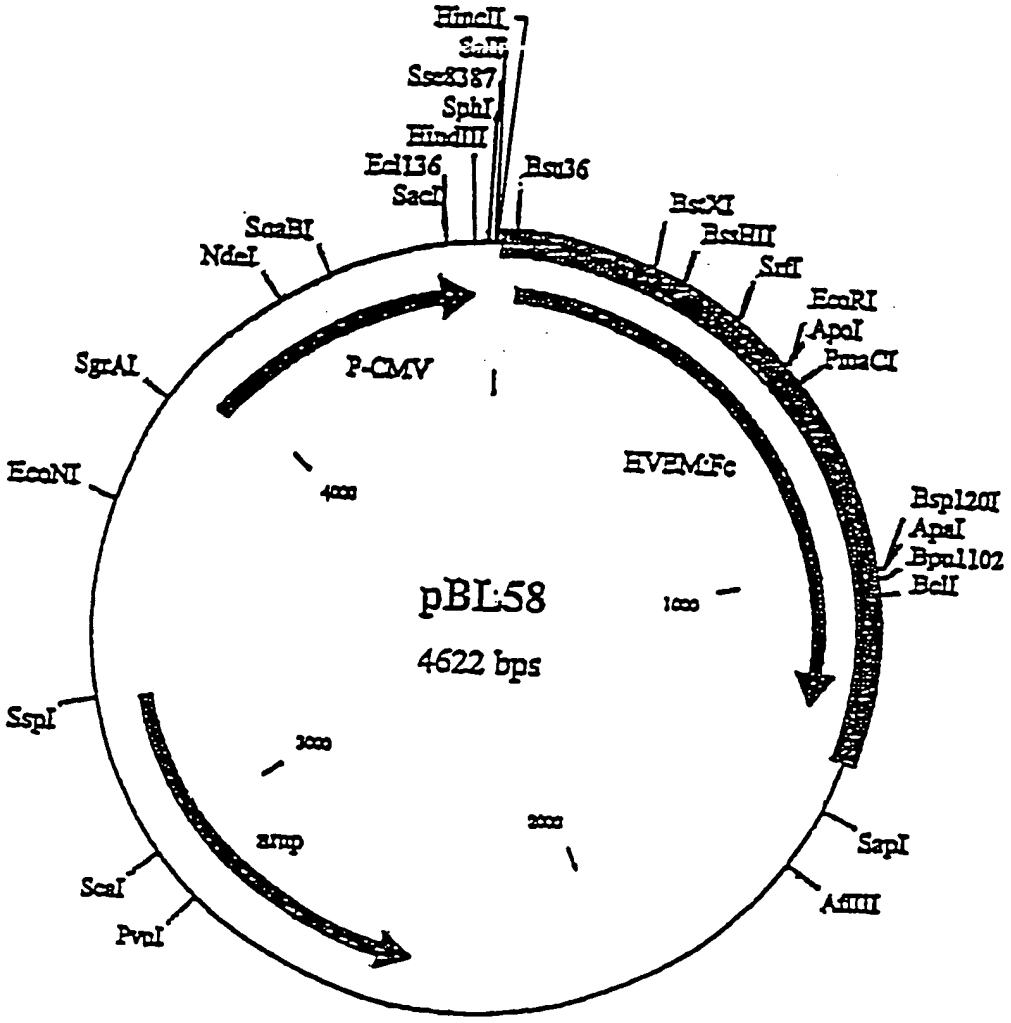

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12374

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.2, 185.1, 204.1, 231.1; 435/6, 7.1, 69.1, 320.1; 530/350, 352, 380; 536/23.1; 935/23, 27, 33, 41, 66, 70, 72

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CAPLUS, EMBASE, BIOTECHDS
search terms: herpes, hsv, vzv, entry, binding, antibodies, receptor, heparan sulfate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	McCLAIN et al. Cell-Specific Kinetics and Efficiency of Herpes Simplex Virus Type 1 Entry Are Determined by Two Distinct Phases of Attachment. Virology. 1994. Vol. 198, pages 690-702, see entire document.	1-27
A	JOHNSON et al. Soluble Forms of Herpes Simplex Virus Glycoprotein D Bind to a Limited Number of Cell Surface Receptors and Inhibit Virus Entry into Cells. Journal of Virology. June 1990, Vol. 64, No. 6, pages 2569-2576, see entire document.	1-27
A	SUBRAMANIAN et al. Swine Testis Cells Contain Functional Heparan Sulfate but Are Defective in Entry of Herpes Simplex Virus. Journal of Virology. Sept 1994, Vol. 68, No. 9, pages 5667-5676, see entire document.	1-27

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 25 OCTOBER 1996	Date of mailing of the international search report 07 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DATQUAN DANNY LEE Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12374

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHIEH et al. Cell Surface Receptors for Herpes Simplex Virus Are Heparan Sulfate Proteoglycans. Journal of Cell Biology. March 1992, Vol. 116, No. 5, pages 1273-1281, see entire document.	1-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/12374

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 63/00; A61K 38/17, 39/00, 39/12, 39/245; C07K 14/035; C12P 21/06; G01N 33/53; C12Q 1/68; C12N 15/12, 15/38

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/93.2, 185.1, 204.1, 231.1; 435/6, 7.1, 69.1, 320.1; 530/350, 352, 380; 536/23.1; 935/23, 27, 33, 41, 66, 70, 72